Bursaphelenchus seani n. sp. (Nematoda: Aphelenchoididae), a phoretic associate of *Anthophora bomboides stanfordiana* Cockerell, 1904 (Hymenoptera: Anthophoridae) *

Robin M. Giblin and Harry K. Kaya

Division of Nematology, University of California, Davis, Ca 95616, U.S.A.

Summary

Bursaphelenchus seani n. sp. is described and illustrated ; its life stages and gonadal development are reported. Growth and development experiments were conducted on monoxenic cultures of the fungus, *Monilinia fructicola*, at 25°. Generation time, from second stage juvenile (J2) to J2 eclosion of the next generation, is four to four days and a half. Developmental time, from dispersal third stage juvenile, dauer (JIII), to J2 eclosion, is four days. The genus *Huntaphelenchoides* Nickle, 1970 is synonomized with *Bursaphelenchus* Fuchs, 1937.

Résumé

Bursaphelenchus seani *n. sp. (Nematoda : Aphelenchoididae) associé phorétique d'*Anthophora bomboides stanfordiana *Cockerell, 1904 (Hymenoptera : Anthophoridae)*

Bursaphelenchus seani n. sp. est décrit et figuré. Son cycle biologique et le développement de ses gonades sont décrits. Les expériences de croissance et de développement ont été effectuées en cultures monoxéniques du champignon *Monilinia fructicola* à 25°. Une génération, du juvénile du second stade (J2) au J2 de la génération suivante, dure de qutre à quatre jours et demie. Le temps de développement, mesuré depuis l'organisme de dispersion, le juvénile de troisième stade (JIII) jusqu'à l'éclosion des J2 est de quatre jours. Le genre *Huntaphelenchoides* Nickle, 1970 est synonymisé avec *Bursaphelenchus* Fuchs, 1937.

The phoretic association between a *Huntaphelenchoides* sp. and the solitary, soil-dwelling bee, *Anthophora bomboides stanfordiana* Cockerell, 1904 (Hymenoptera : Anthophoridae) was reported by Giblin and Kaya (1980) and Giblin, Kaya and Broocks (1981). The nematode, at that time, was identified as *Huntaphelenchoides* sp. based on the spicule shape of the males. Comparisons made between the diagnostic characters of *Huntaphelenchoides* and *Bursaphelenchus* indicate that the genus *Huntaphelenchoides* Nickle, 1970 is a synonym of *Bursaphelenchus* Fuchs, 1937. The nematode in this study and cited in Brooks (1979), Giblin and Kaya (1980) and Giblin, Kaya and Brooks (1981) was determined to be a new species and is described herein as *Bursaphelenchus seani* n. sp. Furthermore, the life cycle, generation time and dauer juvenile development of *B. seani* n. sp. were studied.

Materials and methods

Measurements

Adults of *B. seani* n. sp. were collected from brood cells containing solitary larvae of *A. bomboides stanfordiana* at Bodega Head State Park, Sonoma Co., California on June 22, 1980. These nematodes were heat killed and preserved in 2.5% formalin.

* Submitted in partial fulfillment of the Ph.D. degree for Robin M. Giblin.

Permanent mounts were prepared from specimens processed into glycerol (Southey, 1970). Measurements were taken from the formalin preserved specimens and from the glycerol mounts. Median spicule measurements were made along the chord of the spicule.

Laboratory studies were conducted with *B. seani* n. sp. from Bodega Head State Park cultured on one week old plates of the fungus, *Monilinia fructicola* (Wint.) Honey, on potato dextrose agar (PDA). at 25°. This fungus serves as an excellent host for *B. seant* (Giblin & Kaya, 1983). Body lenght and gonad measurements and illustrations were made of the propagative and dispersal stages of *B. seani;* juvenile propagative stages are designated with arabic numerals, i.e., J2 = propagative second-stage, while the dispersal stages are designated with Roman numerals, i.e., JIII = dispersal third-stage juvenile. Intermolts are designated as hyphenated combinations of the two nematode stages involved in the molt, i.e., J2-JIII is the intermolt between the propagative second-stage and the dispersal third-stage juvenile. All stages of the propagative phase were heat killed, stained in 45% acetic orcein (Hirschmann, 1962) for 24-48 h, processed via Seinhorst fast method to glycerol (in Southey, 1970), and the various intermolt stages were drawn and measured. Measurements of the J2-JIII intermolt were taken from *B. seani* n. sp. cultured at least seven weeks on *M. fructicola* cultures supplemented with glycerol, 115 mg/g hydrated PDA (Giblin & Kaya, 1983). The JIII-J4 intermolts were observed and measured when field collected JIIIs from brood cells were placed in culture for 19-28 h. Dispersal intermolts and stages were heat killed, stained for over 24 h in 45% acetic orcein, and then measured and observed. Measurements of eggs were made from temporary water mounts.

Life cycle studies

In the first study, eggs were washed and separated from cultures into a dish filled with distilled water. Newly-hatched nematodes were collected at 2 h intervals and ten nematodes each were added to individual tissue culture wells (1.7 cm diam. × 1.0 cm high) containing a 0.8 cm diam. core sample of *M. fructicola* mycelia. The nematodes were collected from these core samples at 8-24 h intervals for five days, heat killed, stained in 45% acetin orcein and their development observed.

In the second study, cohorts of ten field-collected JIIIs were placed on fungal mycelia in culture wells and collected and processed for observations as in the first study.

Photomicrographs

Photomicrographs of *B. seani* n. sp. adults were taken with a Nikon automatic 35 mm camera mounted on a Leitz compound microscope with Normarski optics. Scanning electron microscope (SEM) observations were made of cultured adults that were heat killed, fixed with 3% glutaraldehyde in 0.1 M phosphate buffer pH 7.2 for 2 h, and then cut in half and fixed overnight. The fixed adults were then dehydrated in ethanol, processed into amyl acetate, critical point dried, sputter coated with gold and viewed on a Cambridge Stereoscan at 10 KV.

Statistics

Mean of measurements of selected life stages were statistically compared by one way analysis of variance and separation of means was done with a Student-Newman-Keuls Multiple Range test.

Bursaphelenchus seani * n. sp.

(Figs 1 & 2)

Measurements (in 2.5% formalin)

Males (paratypes ; n = 25) : L = 1.03 mm ± 0.16 (0.66-1.29) ; W (width) = 35 µm ± 5 (25-43) ; a = 29 ± 2 (27-33) ; E (distance from anterior end to junction of esophagus and intestine) = 89 µm ± 4 (78-95) ; b = 11.6 ± 1.6 (8.5-13.7) ; tail length = 45 µm ± 8 (22-57) ; c = 23.1 ± 3.7 (18.0-27.3) ; Spicule median length = 23 µm ± 2 (18-27) ; Stylet = 16 µm ± 1 (13-18).

Females (paratypes ; n = 25) : L = 1.21 mm ± 0.13 (0.77-1.35) ; W = 43 µm ± 5 (27-53) ; a = 28 ± 2 (25-32) ; E = 89 µm ± 3 (83-93) ; b = 13.6 ± 1.4 (9.3-15.3) ; tail length = 71 µm ± 7 (54-82) ; c = 17.1 ± 1.3 (14.3-19.0) ; V = 78 (76-82) ; Stylet = 17 µm ± 1 (15-19).

Holotype (male) : L = 0.92 mm ; W = 26 µm ; a = 35 ; E = 93 µm ; b = 9.9 ; tail length = 35 µm ; c = 26.4 ; Spicule median length = 24 µm ; Stylet = 17 µm.

Allotype (female) : L = 1.34 mm ; W = 42 µm ; a = 32 ; E = 99 µm ; b = 12.8 ; tail length = 52 µm ; c = 25.9 ; V = 79% ; Stylet = 18 µm.

* Named in loving memory of Sean Gregory Giblin, brother of R.M.G.

DESCRIPTION

Male : Body cylindrical, annulated, annules about 0.8 µm wide. Lip region offset. Lip annules visible with SEM only ; En face view with light microscope with six lips with inner hexaradiate framework. Lateral lips narrower than sublaterals. Amphidial apertures dorsal to lip midline, at edge of cephalic plate. Cephalic papilla on the outer margin of each sublateral lip. Six outer labial papillae visible but obscure. Six inner labial papillae visible with SEM only. Stylet, two parts ; cone short, shaft with basal thickenings. Procorpus about 2-2½ anal body widths long ending in well-developed metacorpus. Œsophageal-intestinal junction about one metacorpus valve length behind metacorpus. Postcorpus glandular, dorsally located. Excretory pore behind metacorpus, just behind nerve ring, anterior tip of hemizonid about 25 µm behind excretory pore. Gonad outstretched. Lateral field with four incisures, beginning midway between head and start of metacorpus, extending posteriorly to preanal papillae (P2) ; reduced to three incisures at preanal papillae (P2) to bursal flap = caudal ala. Tail about two anal-body widths long, ventrally curved ; terminus claw -like from lateral view. Bursal flap surrounds end of tail, finely striated flap spade-shaped from ventral view. Seven ventral preanal and postanal papillae : one preanal papilla (P1) in ventral midline at level of spicule rostrum, one pair preanal subventral papillae (P2), one pair of subventral postanal papillae (P3) about 2/3 of tail length behind cloaca, one ventral pair of papillae (P4) just anterior to bursal flap. Spicules separate ; rostrum sharply pointed, rostrum plus a transverse bar form ventral element, end of lamina wide and rounded.

Female : Adult male and female cuticular features and cephalic regions similar. Ovary outstretched. Vulva a transverse slit, vulval lips protrude slightly. Paired, three-pronged, cuticular organs at junction of uterus and postvulval sac, similar to structure in *Aphelenchoides polygraphi* Massey, 1974. Parts of vagina very heavily sclerotized. Length of postvulval sac less than 1/2 vulva to anus distance. Some specimens postvulval sac filled with sperm or with J2 in egg. In some older females body width slightly constricted posterior to vulva. Anus dome shaped slit. Tail about 3½ anal body widths long, terminates in sharp cuticular point (Figs. 1E & 2D).

TYPE SLIDES

Holotype (male) : Collected June 22, 1980 by R. M. Giblin. Catalogue NO. UCNC 2003, University of California, Davis. Paratypes (males and females), deposited at University of California, Davis (UCNC 2004-2006) ; USDA Nematode Collection, Beltsville, Maryland ; Nematology Department, Rothamsted Experimental Station, Harpenden, Herts., England ; Lab. voor Nematologie, Binnenhaven 10, Wageningen, Netherlands.

TYPE HABITAT

Associated with a variety of fungi in the brood cells of the soil-dwelling bee, *Anthophora bomboides stanfordiana.*

TYPE LOCALITY

Bluffs at Bodega Head State Park, Sonoma County, California, U.S.A.

DIAGNOSIS

Bursaphelenchus seani n. sp. is close to *B. fungivorus* Franklin & Hooper, 1962, *B. gonzalezi* Loof, 1964 and *Aphelenchoides hunti* Steiner, 1935. Males of the above mentioned species have a bursal flap, as do all males of *Bursaphelenchus*, but can be separated by spicule shape. The spicules for these males are separate and mitten-shaped as are spicules from other members of *Bursaphelenchus*, but differ in possessing a transverse cross bar and a lamina with a wide and rounded tip. Although not previously described, examination of female paratypes of the above mentioned species revealed a pair of three-pronged cuticular organs at the junction of the uterus and the postvulval sac as described for *B. seani* n. sp.

B. seani n. sp., *B. gonzalezi* and *B. fungivorus* males and females possess a lateral field with four incisures and can be differentiated from males and females of *A. hunti* which possess a lateral field with three incisures. Examination of the paralectotypes of *A. hunti* (slides UCNC 1267-1268) at the University of California, Davis confirms Steiner's (1935) description of three incisures for *A. hunti*, Nickle's (1970) observation of a bursal flap on the male and Franklin and Hooper's (1962) observation of two pairs of postanal papillae, instead of three pairs as described by Steiner.

Males and females of *B. seani* n. sp. differ from *B. gonzalezi* in the distance between the excretory pore and the anterior tip of the hemizonid. In *B. seani* n. sp. this distance is about 25 µm, whereas in *B. gonzalezi* the anterior tip of the hemizonid abuts the excretory pore. Males of *B. seani* n. sp. differ from *B. gonzalezi* in that the latter species possesses a pair of dorsal postanal papillae while *B. seani* n. sp. does not.

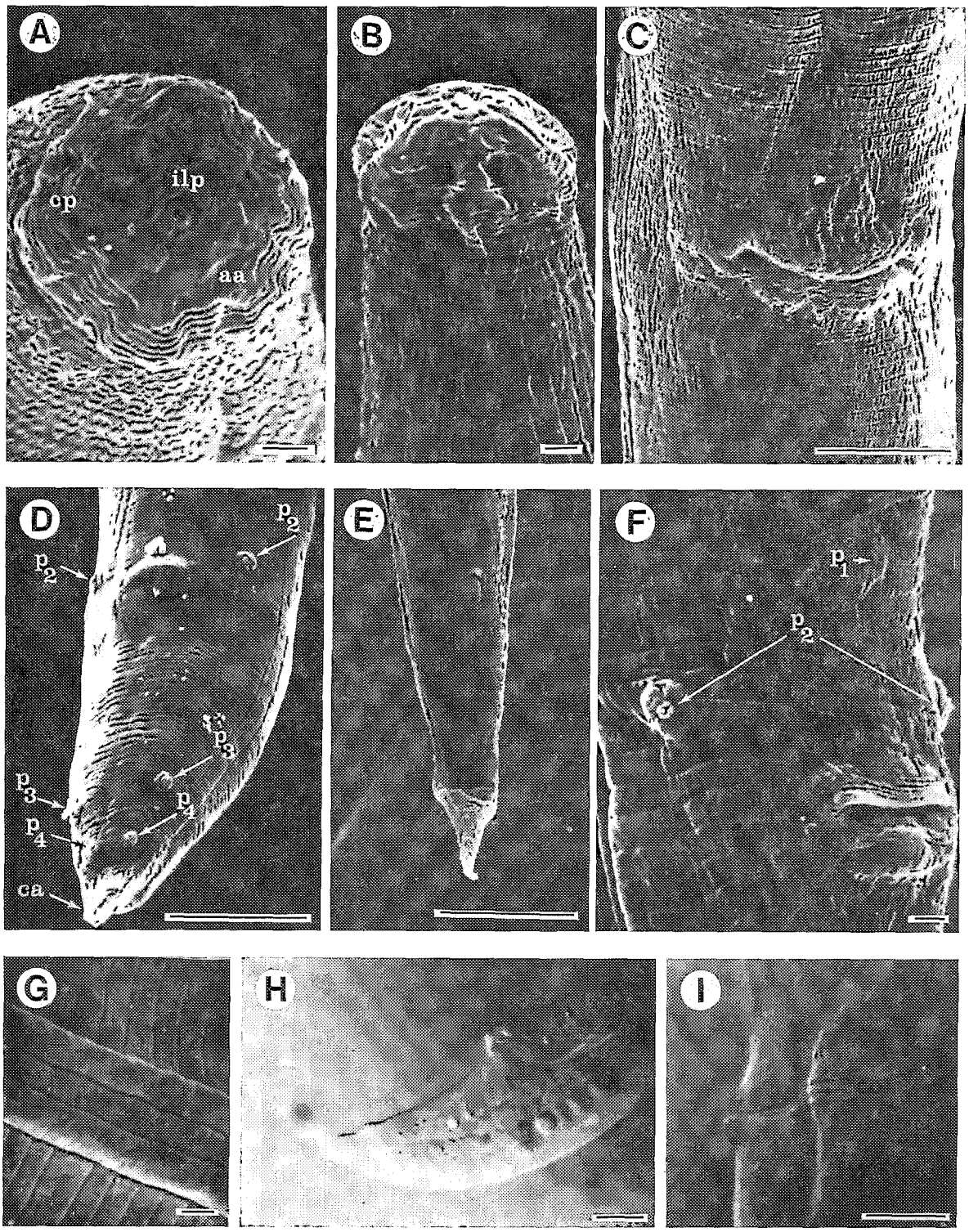

Fig. 1. *Bursaphelenchus seani* n.sp. (A-G, SEM photomicrographs ; H-I, photomicrographs) ; A : Face view ; B : Anterior region (lateral) ; C : Vulva (ventral) ; D : Tail region of male (subventral) ; E : Female tail (ventral) ; F : Preanal papillae and cloaca of male (subventral) ; G : Lateral field (lateral) ; H : Male spicules and tail (lateral) ; I : Female ; vulva (lateral) ; aa : amphidial aperture, ca ; caudal ala = bursal flap, co ; culticular organ, cp ; cephalic papilla, Pl ; single preanal papilla, P2 ; preanal papillae, P3-P4 ; postanal papillae. (A, B, F, G ; bar = 1 μm) (C, D, E, H, I ; bar = 10 μm).

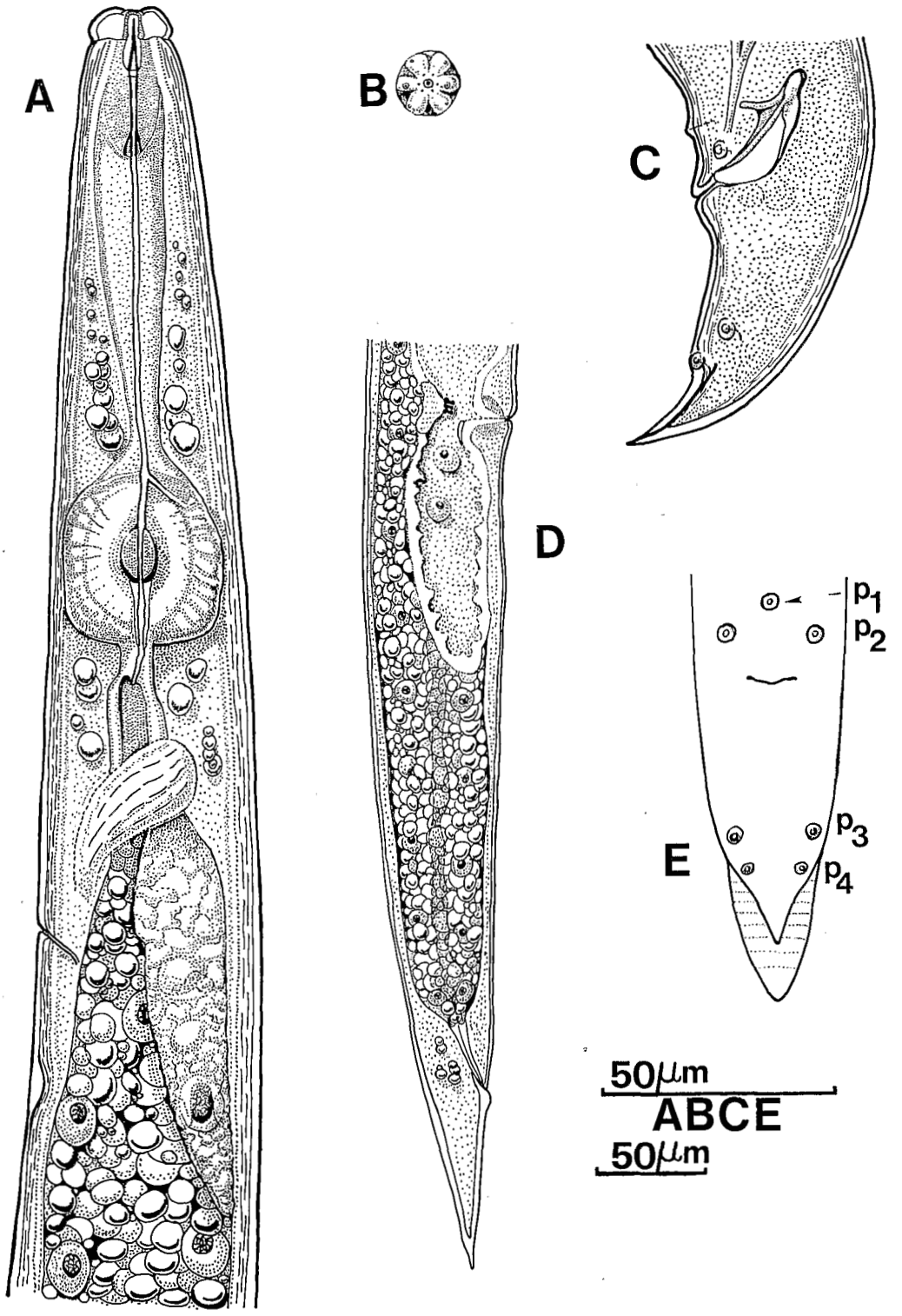

Fig. 2. *Bursaphelenchus seani* n. sp. A : Adult cephalic region and esophagus (lateral) ; B : Face view of head ; C : Male tail with spicules (lateral) ; D : Female body, posterior region (lateral) ; E : Male tail (ventral) (See Fig. 1 for legend).

B. seani n. sp. males are closest to *B. fungivorus*, but can be distinguished in lateral or ventral views by the position of the apex of P2 relative to the position of the apex of P1 and the cloaca. In *B. seani* n. sp. the apex of P2 is usually 50% and always greater than 40% of the distance from the cloaca to the apex of P1, whereas the apex of P2 in *B. fungivorus* is usually at the level of the cloaca and never more than 20% of the distance from the cloaca to the apex of P1. *B. seani* n. sp. females possess a straight, conoid tail that averages 3.4 ± 0.4 anal body-widths long (range = 2.5-4.6 ; n = 25) and terminates in a sharp cuticular point, whereas *B. fungivorus* females possess a tail 6-7 anal body-widths long with a narrow and rounded terminus that bends ventrally when killed.

Life cycle of Bursaphelenchus seani n. sp

Egg

Ten eggs containing J2 juveniles were measured just prior to eclosion. The average length was 71 µm ± 4 (64-78), width = 33 µm ± 2 (31-36), and the length/width ratio was 2.2 ± 0.2 (1.9-2.4).

The first molt (J1-J2) occurs within the egg. This contrasts with the observations of Franklin and Hooper (1962) who reported that *B. fungivorus* eclosed as a J1. In *B. seani* n. sp., the shed cuticle can be seen as a small cap on the head of the J2 during the molt within the egg (Fig. 3A).

Second stage juvenile

Before and after eclosion, the J2 has a characteristically blunt tail and a four-celled genital primordium, consisting of two small somatic cap cells at the anterior and posterior ends and two large germinal cells in the center (Figs. 3A-B). The lip region and stylet of the J2, J3 and J4 are morphologically similar to the adult. The tail shape of the J2 is blunt compared to the uniformly conoid tail of the J3, J4 and adults. At about 28 h, cell divisions in the gonad of both sexes of the J2 and a spicule primordium in J2 males can be observed. The second molt occurs 36-52 h after eclosion. The gonad of the late intermolt (J2-J3) usually consists of four germinal cells (range = 2-6), seven somatic cells (range = 4-9) and two cap cells (Figs. 3G-J). The germinal cell nuclei are larger and stain more lightly than the somatic cell nuclei. The somatic cells of the gonad proliferate posteriorly in females and anteriorly in males as observed by Hirschmann (1962) for *Ditylenchus triformis* Hirschmann & Sasser, 1955 and by Hechler (1963) for *Seinura tenuicaudata* (de Man, 1895), Goodey, 1960. This type of development has not been observed for *B. xylophilus (= lignicolus)* (Ishibashi, Aoyagi & Kondo, 1978) or for *B. fungivorus* (Franklin & Hooper, 1962).

Third stage juvenile

The male gonad continues to grow anteriorly in the third stage, and the germinal and somatic cells become difficult to differentiate with acetic orcein staining. The nuclei of the spicule primordium continue to divide in males. The female gonad grows posteriorly and a small bulge of cells appears at the site of the future vulva. After this, the growth of the female gonad is mostly anterior. The third molt occurs between 48-52 h after eclosion (Figs. 4E-H). Sometimes during the (J3-J4) intermolt the male gonad will begin to make a 180° turn, and in females ventral chord nuclei are often observed.

Fourth stage juvenile

During the fourth stage the male gonad makes a complete 180° turn, continues to grow anteriorly, and grows posteriorly towards the well developed spicule primordium, as observed for *D. triformis* (Hirschmann, 1962) and S. *tenuicaudata* (Hechler, 1963). Before the late fourth stage intermolt, the male gonad differentiates into the vas deferens and testis. The ventral chord nuclei multiply and enlarge in fourth stage females and at the end of the fourth stage the nuclei envaginate to form the vagina. The fourth molt (J4-adult) occurs about 68 h after eclosion. During the intermolt the spicules become visible, first as a pocket and then as the fully formed spicules (Fig. 4J). The vagina becomes visible during the late intermolt in females while the gonad differentiates into the ovary, oviduct, uterus and postvulval sac. The paired, three pronged cuticular organs, opposing the vagina at the junction of the uterus and the postvulval sac, also appear during the late intermolt (Fig. 4 I).

Adult

Adults are found 72-76 h after eclosion. Adults increase in size after the intermolt, which can be seen when comparisons are made between measurements of J4-adults (Table 1) and adults. Eggs are produced within the next 16-20 h (88-96 h after eclosion) and the first J2s appear 96-108 h after

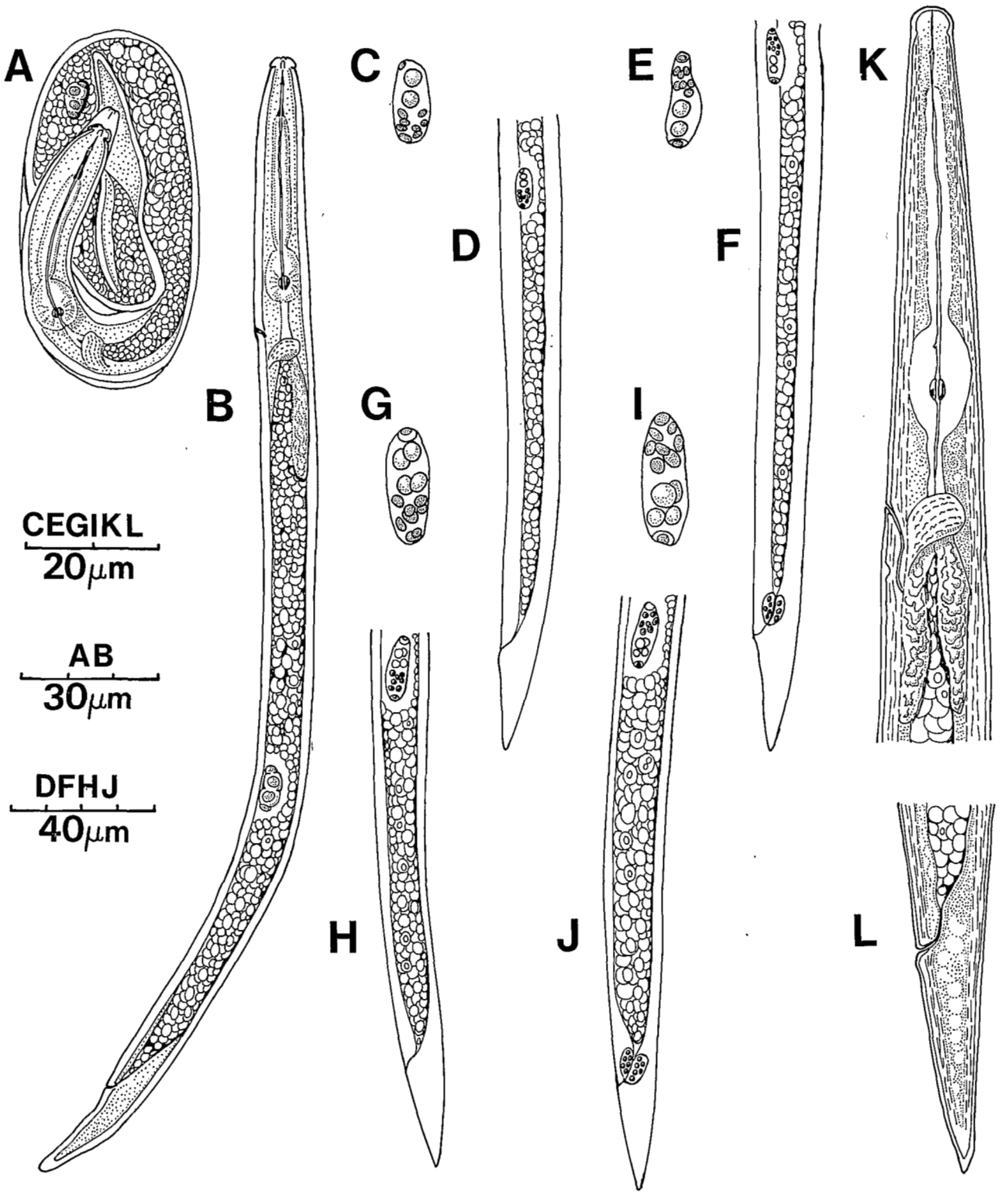

Fig. 3. *Bursaphelenchus seani* n. sp. Life stages (lateral view) ; A : Egg with J2 enclosed within J1 cuticle ; B : J2 ; C : Female J2-JIII gonad ; D : Female J2-JIII midbody and tail ; E : Male J2-JIII gonad ; F : Male J2-JIII midbody and tail ; G : Female J2-J3 gonad ; H : Female J2-J3 midbody and tail ; I : Male J2-J3 gonad ; J : Male J2-J3 midbody and tail ; K : Dauer (JIII) cephalic region and esophagus ; L : Dauer (JIII) tail.

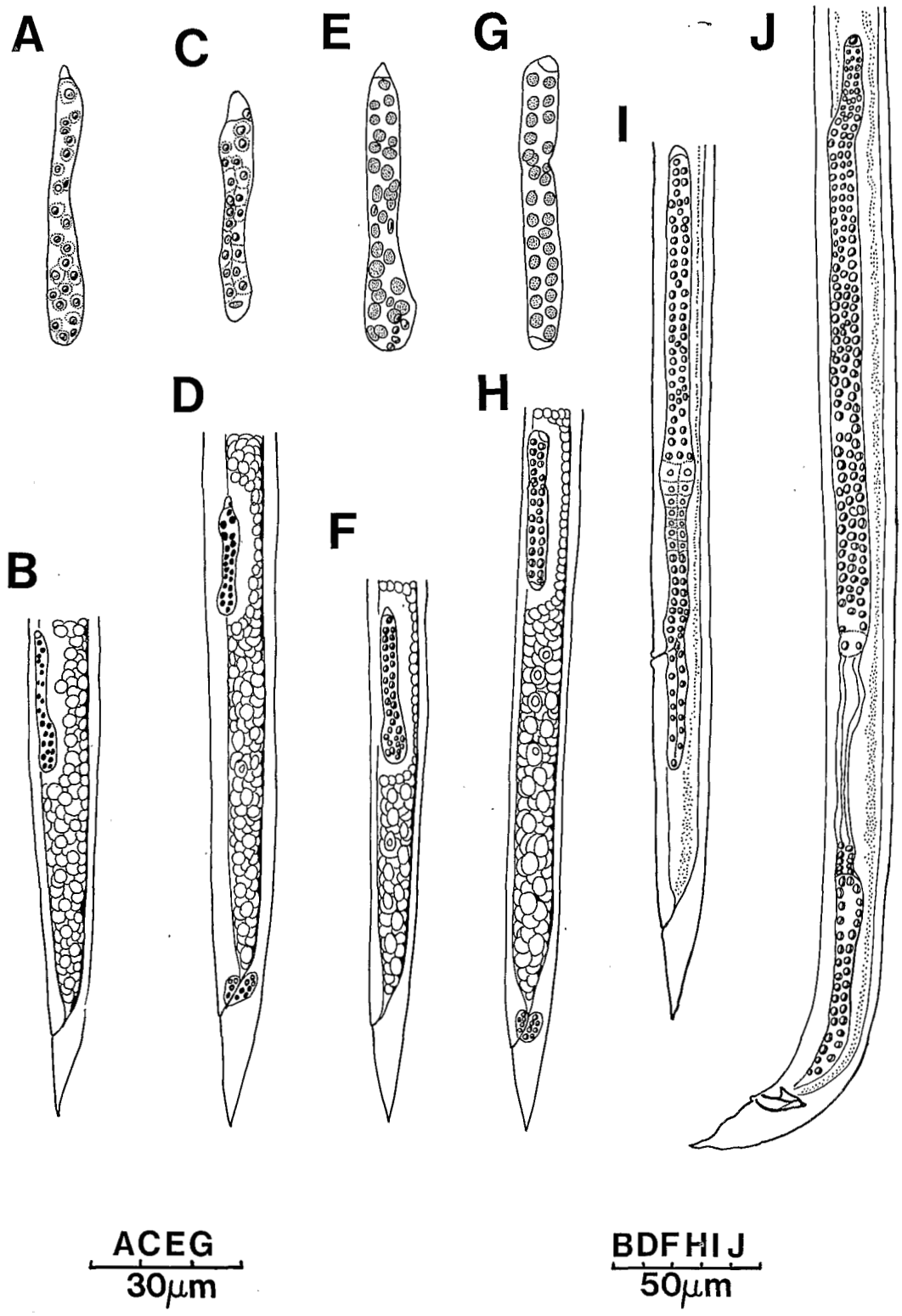

Fig. 4. *Bursaphelenchus seani* n. sp. Life Stages (lateral view) ; A : Female JIII-J4 gonad ; B : Female JIII-J4 midbody and tail ; C : Male JIII-J4 gonad ; D : Male JIII-J4 midbody and tail ; E : Female J3-J4 gonad ; F : Female J3-J4 midbody and tail ; G : Male J3-J4 gonad ; H : Male J3-J4 midbody and tail ; I : Female J4-adult midbody and tail ; J : Male J4-adult midbody and tail.

Table 1

Measurements of intermolts and JIII *Bursaphelenchus seani* n. sp.

Stage		*Body Length*			*Gonad Length*		
	Sex	*X (mm)*	*S.D.*	*Range*	*X (µm)*	*S.D.*	*Range*
J2-J3	M	0.42	0.03	0.37-0.47	23	5	17-34
J2-J3	F	0.39	0.03	0.34-0.44	19	3	16-24
J2-JIII	M	0.59	0.06	0.48-0.72	19	3	14-23
J2-JIII	F	0.57	0.07	0.50-0.65	19	3	12-23
JIII	M	0.54	0.07	0.43-0.63	19	3	14-24
JIII	F	0.54	0.05	0.42-0.61	18	4	12-24
JIII-J4	M	0.52	0.08	0.39-0.66	49	15	32-72
JIII-J4	F	0.51	0.09	0.41-0.65	49	12	31-64
J3-J4	M	0.56	0.05	0.51-0.64	78	39	42-136
J3-J4	F	0.60	0.04	0.55-0.67	87	36	57-172
J4-A	M	0.68	0.06	0.57-0.78	361	91	210-483
J4-A	F	0.80	0.10	0.66-1.00	439	170	227-716

N = 10 for each nematode stage measured ; S.D. = Standard Deviation ; A = Adult.

eclosion of the adults. The life cycle from J2-J2 at 25° on *M. fructicola* takes 4-4.5 days. This relatively fast generation time compares with *B. xylophilus*, which cycles from J2-J2 in about 5 days at 25° on the fungus, *Botrytis cinerea* Pers. *ex* Fr. (Mamiya, 1975).

Dauer juvenile (JIII)

Franklin and Hooper (1962) reported that the J1 of *B. fungivorus* ecloses from the egg, molts to the J2 and then molts to a JIII = J3 stage which was not normally observed until cultures had aged considerably. Our results show that this is not the case for *B. seani* n. sp. There is a normal phase of development with the first molt occurring in the egg, the J2 stage emerges during eclosion and then there are three subsequent molts to the adult. But on older cultures or old cultures supplemented with glycerol some of the J2s grow large, and at the J2-JIII intermolt they are significantly larger ($P < 0.001$) than the normal J2-J3 (see Table 1) and molt to a relatively resistant stage, the dauer juvenile (JIII). This stage is morphologically distinct from the normal J3. The JIII has a dome-shaped head ; the stylet, œsophagus and intestine are indistinct, and the body is filled with granular material (Figs. 3K-L). JIIIs from *A. bomboides stanfordiana* brood cells, reproductive tracts of adult bees and laboratory cultures are morphologically identical. The level of gonad development in the JIII is nearly equivalent to the normal J2-J3 intermolt ; with two cap cells, usually two, three or four germinal cell nuclei (range = 2-6) and about six somatic cell nuclei (range = 4-9) (Figs. 3C-F). JIIIs can be sexed like normal J2-J3 intermolts by the direction of the somatic cell proliferation of the gonad, anterior in males and posterior in females, and also by the presence of a spicule primordium in males. Gonad length is not significantly different ($P > 0.05$) between J2-J3, J2-JIII, and JIII stages. The gonad development in the JIII stage appears to be suspended until the nematodes are placed on fresh fungus cultures of *M. fructicola* for greater than 19 h at 25°, then, just prior to the molt to a J4, the gonadal cells proliferate as in the propagative J3. The third molt occurs 19-28 h after JIIIs were first placed on fresh *M. fructicola*. Franklin and Hooper (1962) observed that the JIII-J4 molt occurred 18 h after JIIIs of *B. fungivorus* were placed on fresh *B. cinerea*. The JIII-J4 intermolts of *B. seani*

n. sp. have a significantly smaller gonad ($P < 0.05$) than the J3-J4 intermolts (see Table 1). Ishibashi, Aoyagi & Kondo (1978) observed differences in gonad lengths between survival and dispersal stages (JIII & JIV) and propagative stages (J3 & J4) of *B. xylophilus*. *B. seani* n. sp. adults appear about 68 h after JIIIs were placed on fresh *M. fructicola* and the first J2s appear less than 27 h later (95 h after introduction of JIIIs onto *M. fructicola*).

The JIII stage of *B. seani* does not feed and is a survival stage. The stylet, esophagus and intestine are weakly developed. There is no significant difference ($P > 0.05$) in the body length between J2-JIII and JIII-J4 intermolts which contrasts with a significant difference ($P < 0.001$) in the lengths between J2-J3 and J3-J4 intermolts (Table 1). The offset lips, distinct stylet, metacorpus and intestine do not appear in the JIII until the late JIII-J4 intermolt. JIII *B. seani* n. sp. from bee cells have been stored in distilled water at 9 ° for 12 months with about 50% survival.

Huntaphelenchoides Nickle, 1970, a minor synonym of **Bursaphelenchus** Fuchs, 1937

Nickle (1970) established *Huntaphelenchoides* as a new genus, designating *Aphelenchoides hunti* Steiner, 1935 as the type and included two species of the genus *Bursaphelenchus* Fuchs, 1937 as new combinations ; *B. fungivorus* Franklin & Hooper, 1962 and *B. gonzalezi* Loof, 1964. The key diagnostic character for the genus is the unique shape of the spicules. Tarjan and Baeza-A. (1982) report that spicule morphology is a primary diagnostic character for *Bursaphelenchus* at the species level. For the purpose of comparison, we have separated several groups within *Bursaphelenchus* according to spicule shape (Fig. 5). These groups are as follows : *B. piniperidae* Fuchs, 1937 (type species), *B. hunti* group, *B. xylophilus* group, *B. eidmanni* group and the *B. eremus* group (Fig. 5A-E). The distinctiveness, "size of gap" (Mayr, 1969), of the spicule morphology between the *B. hunti* group (*B. hunti* (Steiner, 1935) n. comb., *B. fungivorus*, *B. gonzalezi* and *B. seani* n. sp.) and the other groups and members of the genus is not sufficiently different to warrant separate genera. Most males of *Bursaphelenchus* have separate, rosethorn or mitten-shaped spicules, as represented in Fig. 5A by *B. piniperidae*. Males of the *B. hunti* group have separate, mitten-shaped spicules with a prominent rostrum and transverse bar making up the ventral element and a lamina that ends in a wide and rounded tip. Females of this group have no vulval flap.

Males of the *B. xylophilus* group (*B. xylophilus* (Steiner & Buhrer, 1934) Nickle, 1970, *B. mucronatus* Mamiya & Enda, 1979 and *B. fradulentus* (Rühm, 1956) Goodey, 1960) have large, paired, arcuate spicules with a sharply pointed rostrum and a disc-like expansion, cucullus (Tarjan & Baeza-A., 1982), at the distal tip (Fig. 5C). Females of this group have a vulval flap.

Males of the *B. eidmanni* group (*B. eidmanni* (Rühm, 1956) Goodey, 1960 and *B. trilunculus* Massey, 1974) have spicules that appear fused proximally (Figs. 5D-E), and both have similarly shaped bursal flaps. Females of the group have no vulval flap.

Males of the *B. eremus* group (*B. eremus* (Rühm, 1956) Goodey, 1960, *B. cryphali* (Rühm, 1956) Goodey, 1960, *B. bestiolus* Massey, 1974, *B. silvestris* (Lieutier & Laumond, 1978) Baujard, 1980 and *B. leoni* Baujard, 1980) have characteristic spicules

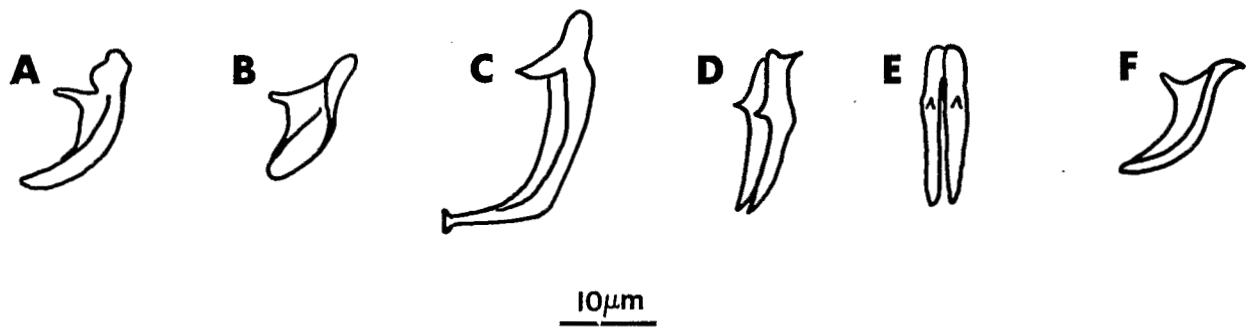

Fig. 5. Spicules of *Bursaphelenchus* (A, B, C, D & F, lateral views ; E. ventral view) ; A : *Bursaphelenchus piniperidae* Fuchs, 1937 (after Rühm, 1956) ; B : *B. hunti* (after Nickle, 1970 ; C : *B. xylophilus* (after Nickle, 1981) ; D-E : *B. eidmanni* (after Rühm, 1956) ; F : *B. eremus* (after Rühm, 1956).

with a dorsal thorn-like projection, condylus (Tarjan & Baeza-A., 1982), that can curve back dorsally (Fig. 5F). Some females of the group have a vulval flap.

There are numerous similarities among the above mentioned groups that support the inclusion of *Huntaphelenchoides* within the genus *Bursaphelenchus*. Females of the *B. hunti* group (= *Huntaphelenchoides*) are nearly indistinguishable at the generic level from most of the females of *Bursaphelenchus* and *Aphelenchoides* Fuchs, 1937. Length of the stylet in the *B. hunti* group ranges from 12-19 µm and is within the range of *Bursaphelenchus* stylet lengths, 11-18 µm ; vulva position in the *B. hunti* group ranges from 68-83% and fits within the range of *Bursaphelenchus*, 64-88% ; median spicule length in the *B. hunti* group males ranges from 17-24 µm, which is within the range for *Bursaphelenchus*, 11-31 µm ; body lengths and ratios (a, b, c) for the *B. hunti* group coincide with variation of lengths and ratios found in *Bursaphelenchus*. Female tail shapes and lengths are variable for all groups.

Comparisons of SEM micrographs of the head region of *B. fungivorus* (Hooper & Clark, 1980), *B. xylophilus* (Nickle *et al.*, 1981 ; Yik & Birchfield, 1981) and *B. seani* n. sp. (Figs. 1 A-B) illustrate that the above mentioned species have the same basic "aphelenchoidid" cephalic plate pattern, with prominent lips, visible amphidial apertures, four cephalic papillae and six inner labial papillae. Hooper and Clark (1980) observed outer labial papillae on *B. fungivorus* and these were occasionally visible on *B. seani*, but were difficult to see. The micrographs of *B. xylophilus* are not clear enough to discern whether the outer labial papillae open onto the lips or not. Males of the *B. hunti* group appear to have seven ventral preanal and postanal papillae. The recent observations of seven ventral papillae on males of *B. xylophilus* by Yik and Birchfield (1981) and Nickle *et al.* (1981) and the seven ventral papillae reported for *B. bakeri* Rühm, 1964 (Rühm, 1960) indicate similarities between the *B. hunti* group and other groups within *Bursaphelenchus*.

We conclude that *Huntaphelenchoides* Nickle, 1970 is a minor synonym of *Bursaphelenchus* Fuchs, 1937. The emended diagnosis as proposed by Baujard (1980) for the genus *Bursaphelenchus* requires no changes.

ACKNOWLEDGEMENTS

We thank Drs. A. R. Maggenti, D. J. Raski, R. Fortuner, A. C. Tarjan and Ms. E. M. Noffsinger for reading the manuscript, Dr. A. C. Tarjan for communications and providing an advance copy of his unpublished paper and Ms. E. M. Noffsinger and Drs. P. A. A. Loof and D. J. Hooper for lending us type specimens of *Bursaphelenchus hunti*, *B. gonzalezi*, and *B. fungivorus*, respectively for examination and comparison. Special thanks to the personnel of Bodega Head State Park, Sonoma Co., Calif. for their cooperation in this study.

RÉFÉRENCES

BAUJARD, P. (1980). Trois nouvelles espèces de *Bursaphelenchus* (Nematoda : Tylenchida) et remarques sur le genre. *Revue Nématol.*, 3 : 167-177.

BROOKS, R. (1979). *The Systematics and Ecology of the* Anthophora bomboides *Species Group in North America.* University of California, Davis., M.Sc. Thesis.

FRANKLIN, M. T. & HOOPER, D. J. (1962). *Bursaphelenchus fungivorus* n. sp. (Nematoda : Aphelenchoidea) from rotting gardenia buds infected with *Botrytis cinerea* Pers. Ex Fr. *Nematologica*, 8 : 136-142.

FUCHS, A. G. (1937). Neue parasitische und halbparasitische Nematoden bei Borkenkäfern und einige andere Nematoden. I. Teil. Die Parasiten der Waldgärtner *Myelophilus piniperda* L. und minor Hartig und die Genera *Rhabditis* Dujardin, 1845 und *Aphelenchus* Bastian, 1865. *Zool. Jahrb., Syst.*, 70 : 291-380.

GIBLIN, R. M. & KAYA, H. K. (1980). The association of a nematode, *Huntaphelenchoides* sp. (Aphelenchoididae) with the solitary soil dwelling bee, *Anthophora bomboides stanfordiana* (Anthophoridae : Hymenoptera) (Abstract). *J. Nematol.*, 12 : 221.

GIBLIN, R. M. & KAYA, H. H. (in prep.). Host, temperature and media additive effects on the growth of *Bursaphelenchus seani*.

GIBLIN, R. M., KAYA, H. K. & BROOKS, R. W. (1981). Occurrence of *Huntaphelenchoides* sp. (Aphelenchoididae) and *Acrostichus* sp. (Diplogasteridae) in the reproductive tracts of soil nesting bees (Hymenoptera : Apoidea). *Nematologica*, 27 : 20-27.

GOODEY, J. B. (1960). The classification of the Aphelenchoidea. *Nematologica*, 5 : 111-126.

HECHLER, H. C. (1963). Description, developmental biology, and feeding habits of *Seinura tenuicaudata* (de Man) J. B. Goodey, 1960 (Nematoda : Aphelenchoididae), a nematode predator. *Proc. helminth. Soc. Wash.*, 30 : 182-195.

HIRSCHMANN, H. (1962). The life cycle of *Ditylenchus triformis* (Nematoda : Tylenchida) with emphasis on post-embryonic development. *Proc. helmint. Soc. Wash.*, 29 : 30-43.

HIRSCHMANN, H. & SASSER, J. N. (1955). On the occurrence of an intersexual form in *Ditylenchus triformis* n. sp. (Nematoda Tylenchida). *Proc. helminth. Soc. Wash.*, 22 : 115-123.

HOOPER, D. J. & CLARK, S. A. (1980). Scanning electron micrographs of the head region of some species of Aphelenchoidea (Aphelenchina : Nematoda). *Nematologica*, 26 : 47-56.

ISHIBASHI, N., AOYAGI, M., & KONDO, E. (1978). Comparison of the gonad development between the propagative and dispersal froms of the pine wood nematode, *Bursaphelenchus lignicolus* (Aphelenchoididae). *Jap. J. Nematol.*, 8 : 28-31.

LIEUTIER, F. & LAUMOND, C. (1978). Nématodes parasites et associés à *Ips sexdentatus* et *Ips typographus* (Coleoptera, Scolytidae) en région parisienne. *Nematologica*, 24 : 184-200.

LOOF, P. A. A. (1964). Free-living and plant-parasitic nematodes from Venezuela. *Nematologica*, 10 : 201-300.

MAMIYA, Y. (1975). The life history of the pine wood nematode, *Bursaphelenchus lignicolus*. *Jap. J. Nematol.*, 5 : 16-25.

MAMIYA, Y. & ENDA, N. (1979). *Bursaphelenchus mucronatus* n. sp. (Nematoda : Aphelenchoididae) from pine wood and its biology and pathogenicity to pine trees. *Nematologica*, 25 : 353-361.

MASSEY, C. L. (1974). *Biology and Taxonomy of Nematode Parasites and Associates of Bark Beetles in the United States*. U. S. Government Printing Office, Wash. D. C., 233 p.

MAYR, E. (1969). *Principles of Systematic Zoology*. New York, McGraw-Hill : 428 p.

Accepté pour publication le 2 mai 1982.

NICKLE, W. R. (1970). A taxonomic review of the genera of the Aphelenchoidea (Fuchs, 1937) Thorne, 1949 (Nematoda : Tylenchida). *J. Nematol.*, 2 : 375-392.

NICKLE, W. R., GOLDEN, A. M., MAMIYA, Y., & WERGIN, W. P. (1981). On the taxonomy and morphology of the pine wood nematode, *Bursaphelenchus xylophilus* (Steiner & Buhrer 1934) Nickle 1970. *J. Nematol.*, 13 : 385-392.

RÜHM, W. (1956). Die Nematoden der Ipiden. *Parasitol. Schriftenr.*, 6 : 437 p.

RÜHM, W. (1960). Ein Beitrag zur Nomenklatur und Systematik einiger mit Scolytiden vergesellschafteter Nematodenarten. *Zool. Anz.*, 164 : 201-213.

RÜHM, W. (1964). Ein Beitrag zur Vergesellschaftung zwischen Nematoden und Insekten (*Pelodera bakeri* n. sp. [Nematoda, Rhabditoidea, Rhabditidae] eine mit *Calvertius tuberosus* Perm. et Germ. [Coleoptera, Curculionidae, Hylobiinae] vergesellschaftete Nematodenart an *Araucaria araucana* [Mol.] Koch). *Zool. Anz.*, 173 : 212-220.

SOUTHEY, J. F. (1970) (Ed.). *Laboratory Methods for Work with Plant and Soil Nematodes*. London, Her Majesty's Stationery Office. 148 p.

STEINER, G. (1935). Opuscula miscellanea nematologica, II. *Proc. helminth. Soc. Wash.*, 2 : 104-110.

TARJAN, A. C. & BAEZA-A., C. (1982). An analysis of the genus *Bursaphelenchus* Fuchs, 1937. *Nematropica*, 12 : 121-144.

YIK, CHOI-PHENG & BIRCHFIELD, W. (1981). Observations on the morphology of the pine wood nematode, *Bursaphelenchus xylophilus*. *J. Nematol.*, 13 : 376-384.